

Phylogenetic Analysis of MERS-CoV in a Camel Abattoir, Saudi Arabia, 2016–2018

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We detected Middle East respiratory syndrome coronavirus (MERS-CoV) RNA in 305/1,131 (27%) camels tested at an abattoir in Al Hasa, Eastern Province, Saudi Arabia, during January 2016–March 2018. We characterized 48 full-length MERS-CoV genomes and noted the viruses clustered in MERS-CoV lineage 5 clade B.

Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) is a zoonotic disease of concern for global public health (1,2). Dromedary camels are the source of zoonotic infection (3). During 2016–2018, a total of 80 full-length MERS-CoV genome sequences were available from human infections in the Arabian Peninsula where all zoonotic disease has occurred, but only 30 sequences from dromedary camels were available, highlighting the need for contemporary dromedary MERS-CoV sequence data.

During November 2015–June 2018, nasal and rectal swab specimens were collected, typically on a monthly basis, from dromedary camels slaughtered at an abattoir and camel market complex in Al Hasa, Eastern Province, Saudi Arabia (Appendix, <https://wwwnc.cdc.gov/EID/article/26/12/19-1094-App1.pdf>). Most camels for slaughter were bred locally, but some camels were imported from Somalia or Sudan for slaughter. Imported camels came through the port of Jeddah, usually via a large central camel market in Riyadh.

Nasal and rectal swab specimens were collected from 1,131 camels; 4–143 camels were sampled each month. Overall, 288 (25.5%) nasal and 85 (7.5%) rectal swabs were MERS-CoV-positive as confirmed by reverse transcription PCR (RT-PCR; Appendix); cycle threshold values ranged from 15.3 to 39.1 (median 33.6). Most (68/85; 80%) positive rectal swab

specimens were collected from animals that also had a positive nasal swab. Overall, 305 (27%) camels sampled were MERS-CoV-positive from either nasal or rectal swabs. Despite regular exposure to infected camels, none of the abattoir workers had diagnosed clinical MERS disease.

MERS-CoV-positive samples were detected during most months in which samples were tested. Age, sex, date of sampling, and breed data were available for 847 camels. Among animals for which age and sex data were available, RT-PCR positive rates for MERS-CoV were not statistically significantly different by age or sex. Among local camels, MERS-CoV-positive rates by breed were 81/227 (35.7%) Magaheem, 19/87 (21.8%) Sofor, and 27/158 (17.1%) Wodaoh. Among imported camels, 21/146 (14.4%) from Somalia and 64/221 (29%) from Sudan were MERS-CoV-positive.

We obtained 48 full genomes of MERS-CoV from the camel samples; dates of sampling were available for 35 (GenBank accession nos. MN654970–5017). We did not detect evidence of deletions in accessory or other genes. Our newly generated virus genomes phylogenetically clustered within the recombinant lineage 5 clade, a novel recombinant clade that has become progressively dominant in Saudi Arabia since 2014 (4,5) (Appendix Figure). The 48 sequences in this study appear to cluster into 2 groups, which we named group A and group B for ease of description rather than a formal taxonomic designation (Figure). Other sublineages within lineage 5 appear to have gone extinct with no human or animal viruses detected since 2016. Virus group A had viruses sampled from 2014–2017, whereas group B had viruses sampled in 2014–2018. Both virus groups cocirculated in the region during the study period (Figure).

Genetically identical viruses were collected mostly during the same sampling period, suggesting cross-infection in the market. However, identical viruses sometimes were from samples collected 1 month apart, such as SA2557 and SA2626, or 3 months apart, such as SA2199, SA2159, and SA2247, suggesting reintroduction of viruses from the same herd or area into the abattoir at different times. Although we sampled imported camels from Somalia and Sudan, the viruses we detected were clade B lineage 5 viruses rather than the clade C viruses that are known to be enzootic in Africa (6). In several instances, viruses from camels from Sudan (for example, SA4104/2017 in December 2017 or SA2687/2017 in May 2017) were almost identical to viruses concurrently detected in camels from Saudi Arabia, indicating likely cross-infection in the camel market. Virus cross-infection and amplification in the camel market could explain the high overall MERS-CoV-positive rate in the abattoir.

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A virus from a patient from Saudi Arabia who had diagnosed MERS in the United Kingdom in August 2018 (MERS-CoV_England-KSA_1/2018-08-22) was found to be closely genetically related (99.81%

similarity) to a camel virus sampled during this study in 2018 (Figure). As previously reported, viruses from camels and humans interleave within the phylogenetic tree (7), suggesting that viruses in

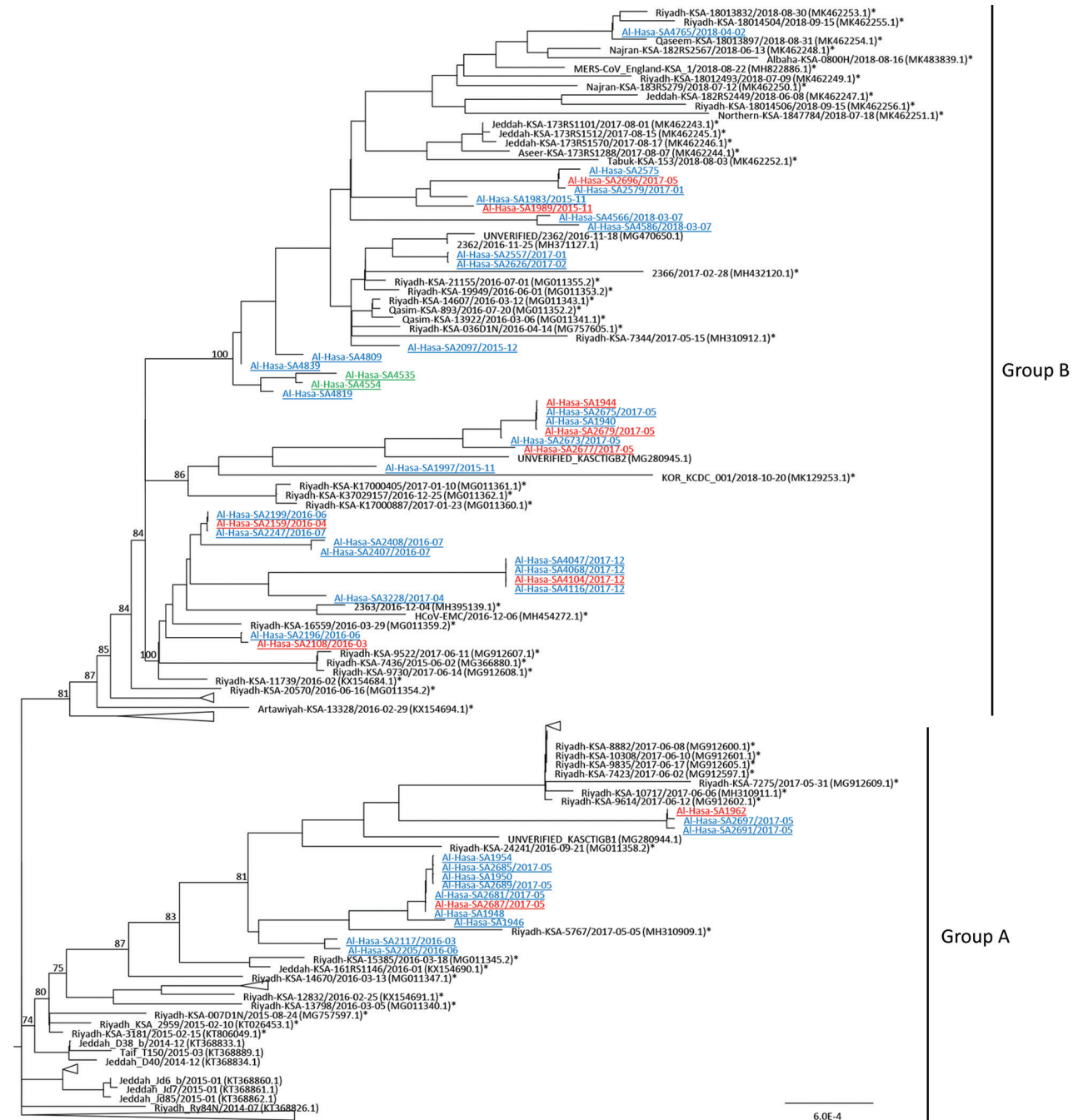


Figure. Phylogeny of Middle East respiratory syndrome coronavirus (MERS-CoV) sequenced from nasal and rectal samples collected from camels in an abattoir, Saudi Arabia. Phylogeny was constructed by using IQTREE (<http://www.iqtree.org>) with the automatic nucleotide transition model selection. Branch supports, shown at major nodes, were generated by ultrafast bootstrap approximation (Appendix, <https://wwwnc.cdc.gov/EID/article/26/12/19-1094-App1.pdf>). Genomes generated from this study are underlined; asterisks (*) indicate viruses from humans. Blue indicates viruses from camels from Saudi Arabia; red indicates viruses in camels imported from Sudan; green indicates viruses in camels imported from Somalia. The overall topology of the phylogeny of MERS-CoV also is available (Appendix Figure). Scale bar indicates 10^4 mutations per site.

camels continue to be the source of human infections through separate zoonotic transmission events without sublineage separation between viruses in camels and humans.

In conclusion, our study suggests multiple lineage 5 clade B viruses continue to be dominant among camels in eastern Saudi Arabia. Camels imported from Sudan and Somalia also had evidence of MERS-CoV B lineage 5 clade viruses prevalent in the Arabian Peninsula, rather than clade C viruses known to be enzootic in camels in Africa. These data suggest imported camels likely acquired MERS-CoV after arriving in Saudi Arabia and that lineage 5 viruses have the greater evolutionary fitness and appear to outcompete other viral lineages, which is concordant with other recently reported data (8). The high rates of MERS-CoV we detected and viral phylogeny suggest likely cross-transmission of MERS-CoV within the camel market and abattoir complex, even among imported animals.

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One-Year Retrospective Review of Psychiatric Consultations in Lassa Fever, Southern Nigeria

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We conducted a retrospective review of psychiatric consultations for hospitalized patients with Lassa fever in southern Nigeria. Ten (8.8%) of 113 patients had psychiatric consultations. Delirium was the most common psychiatric manifestation complicating Lassa fever. Findings suggest that psychiatric intervention could improve overall outcomes of Lassa fever.

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Appendix

Additional Methods

Nasal and rectal swabs were collected, typically on a monthly basis, from dromedary camels slaughtered at an abattoir in Al Hasa, Eastern Province, Saudi Arabia during November 2015–May 2018. Samples were collected according to the animal ethics protocols of the National Committee of Bio-Ethics, King Abdul-Aziz City of Science and Technology, by Royal Decree no. M/59.

Camels for slaughter were sourced from the adjacent large market-complex, which covers 2.3 km² and is divided between sheep and camel pens, auction areas, the slaughterhouse, and animal feed barns. The complex holds ≈5,000 camels at any time in >600 camel pens, each of which holds a few to >40 animals. Camels traded at the market include those for slaughter for meat, milk production, personal collections, genetic stock improvements, and for Mezaeen, camel beauty contests. Most camels in the market are of breeds local to Saudi Arabia and largely are sourced from the eastern and central regions of the country. Imported camels for slaughter came from Somalia or Sudan and came through the port of Jeddah, usually through a large central camel market in Riyadh. Camels remain in the market for a few days to several months. Given proximity and movement of fomites and persons, ample opportunities for cross-transmission of Middle East respiratory syndrome coronavirus (MERS-CoV) between camel pens exists. Each day, 20–40 camels are slaughtered at the abattoir; the numbers vary by seasons and demand. Abattoir workers were male, do not use masks or gloves, but sometimes wear boots and aprons.

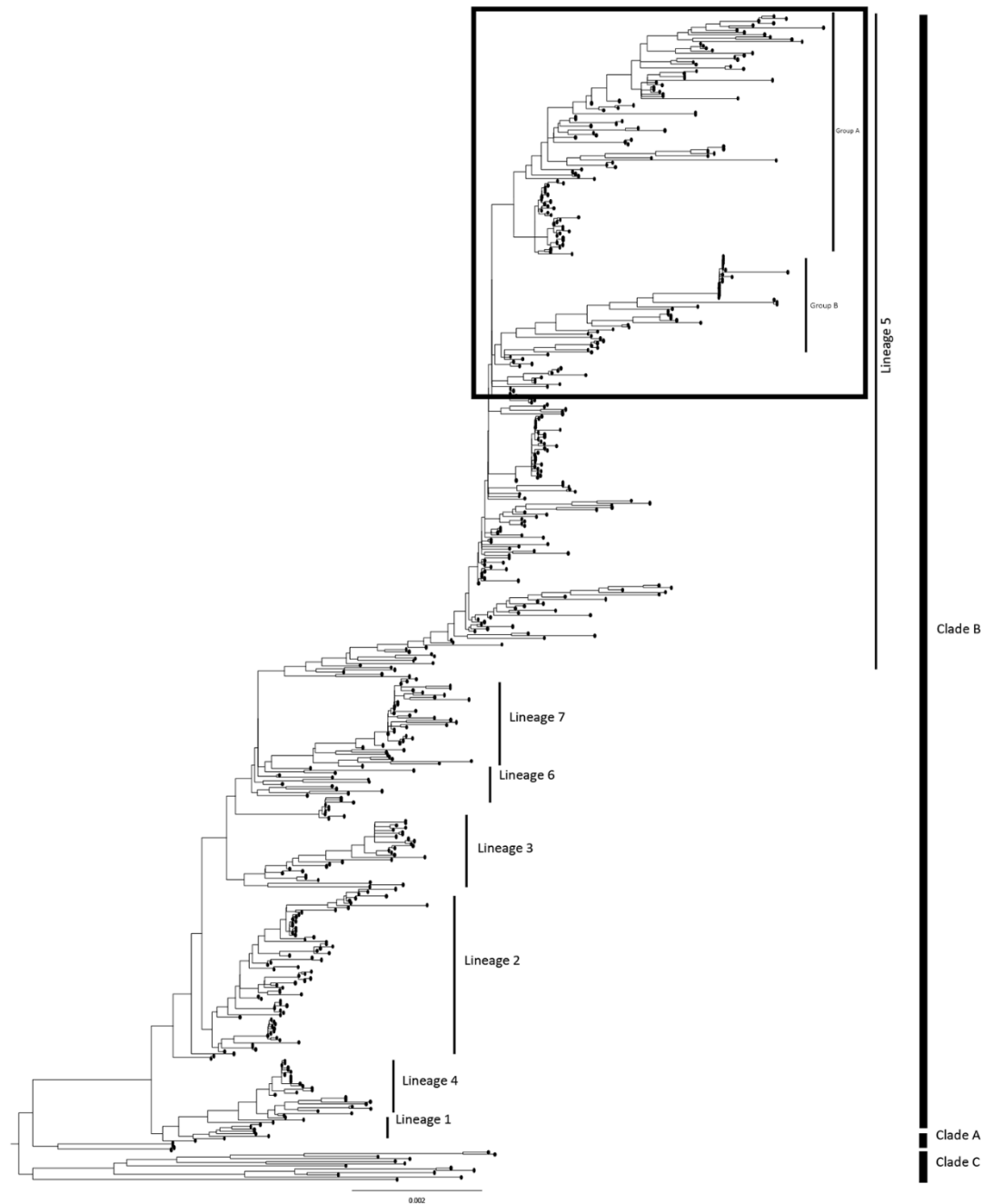
Methods used for specimen collection, virus nucleic acid extraction, quantitative reverse transcription-PCR (qRT-PCR) for virus RNA detection, upE gene confirmed by open reading

frame 1a (ORF1a) gene targets, were described previously (1,2). Samples with upE qRT-PCR cycle threshold (Ct) values <30 were subjected to virus whole-genome sequencing by generating cDNA with gene specific primers and RT-PCR for 2–4 kb overlapping virus genome regions, as previously described (1). PCR products of the genomes were sequenced by using the Solexa (Illumina, <https://www.illumina.com>) sequencing platform with Nextera (Illumina) library preparation method. Virus genomes were assembled with ≥ 100 folds of sequencing coverage.

All available human and camel MERS-CoV genome sequences from the Middle East and Arabian Peninsula (n = 459) and representative sequences from Africa >25.6 kb (85% of the full genome, n = 482) were downloaded from Genbank (<https://www.ncbi.nlm.nih.gov/genbank>) for phylogenetic analysis. Sequences were aligned using MAFFT (<https://mafft.cbrc.jp/alignment/software>) and phylogeny was constructed by using IQTREE (<http://www.iqtree.org>) with the automatic nucleotide transition model selection and ultrafast bootstrap approximation.

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Appendix Figure. Phylogenetic tree of Middle East respiratory syndrome coronavirus (MERS-CoV) detected in humans and camels. Clades and lineages of MERS-CoV are indicated. The area within the box encompasses viruses sequenced in this study. Phylogeny was constructed using IQTREE (<http://www.iqtree.org>) with the automatic nucleotide transition model selection.